CDK4 regulates the myelin sheath of hypothalamic neurons

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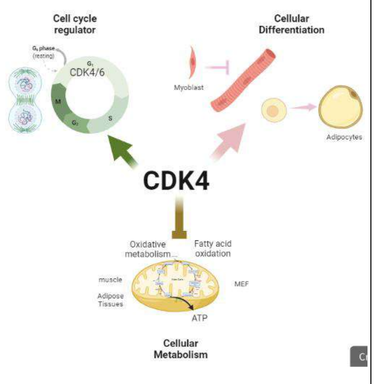
Background

Through autonomic and endocrine functions, the hypothalamus is the control center for many homeostatic mechanisms such as growth, food intake, all-body metabolism, and stress. Recent data obtained by our group and others suggested that **Cyclin-dependent Kinase 4 (CDK4) could be a key factor in the regulation of hypothalamic regulation**. Inhibition of CDK4 expression and/or activity in the hypothalamus altered fat mass gain as well as cold resistance in mice ([1,2]).

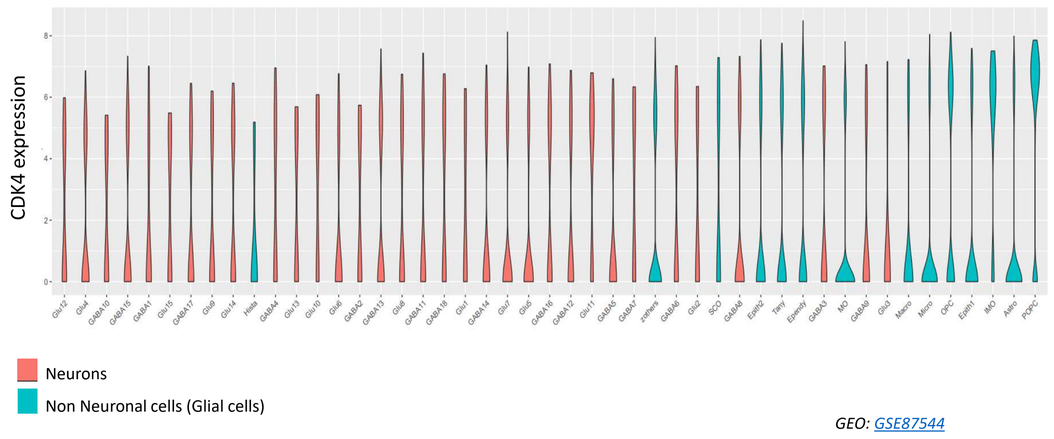
CDK4 is a critical cell cycle regulator controlling G1/S transition. Beyond its role in cell cycle regulation, cumulative evidence has shown non-canonical roles in cell differentiation and regulators of metabolic processes. **However, the role(s) and function(s) of CDK4 in the brain, even more in the hypothalamus, are not well known.**



The objectives of this study are to determine the cell types that expressed CDK4 in the adult hypothalamus, as well as the role of this protein in hypothalamic functions.

[1] J. Castillo-Armengol et al EMBO Reports 2020
[2] N.J. Iqbal, JCI Insight 2018

I- Cdk4 is expressed mainly by glial cells in adult hypothalamus



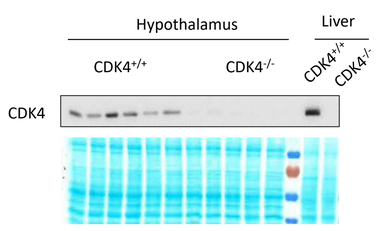
Neurons
Non Neuronal cells (Glial cells)

GEO: GSE87544
Results extrated in Chen et al, Cell Reports 2017

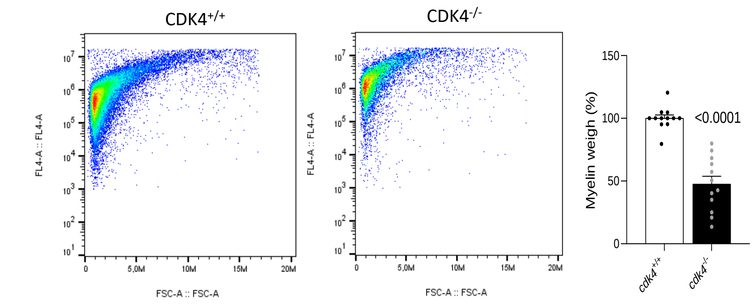
II- Cdk4 deletion impacts hypothalamic myelin content

CDK4 Full KO mice

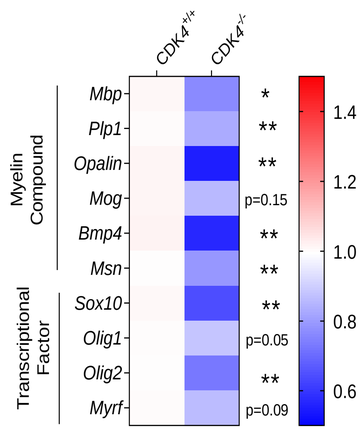
Males – 4 at 5 months old



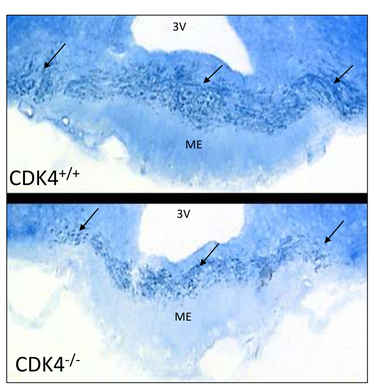
Myelin isolated from hypothalamus using magnetic beat sorting - FACS analysis

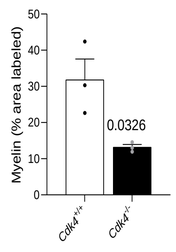


Relative mRNA expression Myelin biosynthesis and compounds



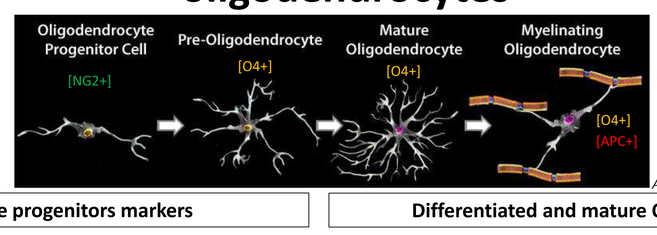
Myelin coloration Blue Fast Luxol coloration





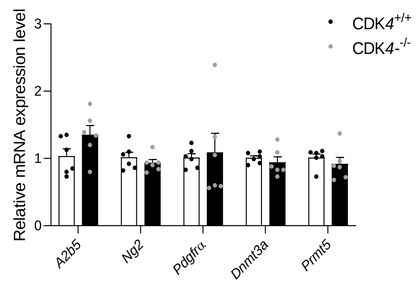
3V: Third Ventricle
ME: Median Eminence

IV- Cdk4 deletion do not seems to impact ontogenesis of oligodendrocytes

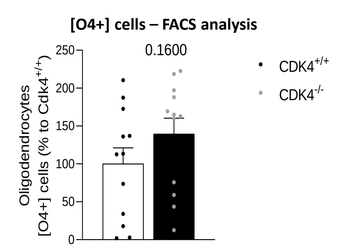


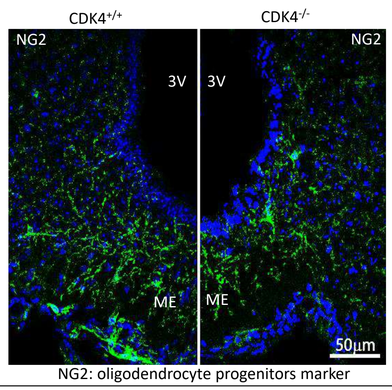
Adapted from S, Kuhn, Cells 2019, *8*(11), 1424

Oligodendrocyte progenitors markers



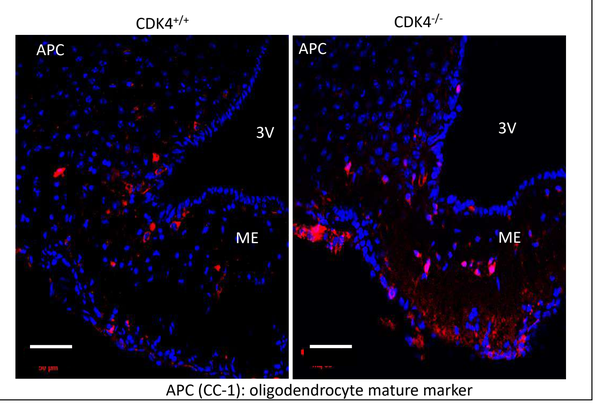
Differentiated and mature Oligodendrocytes markers





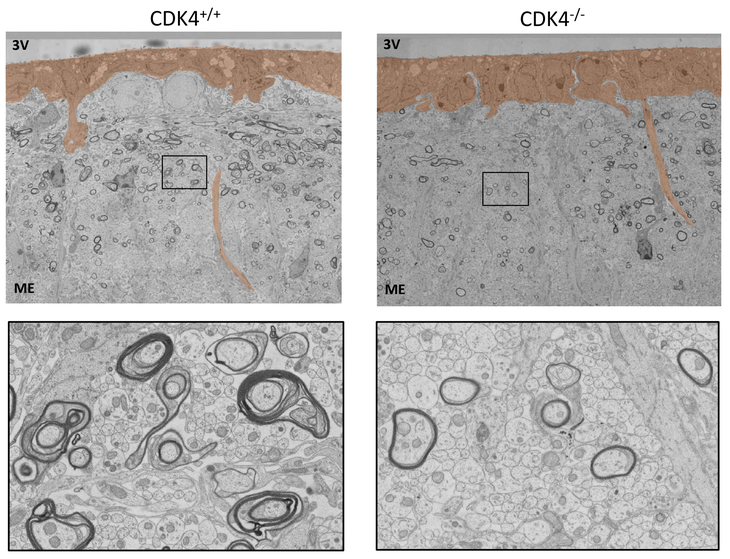
NG2: oligodendrocyte progenitors marker

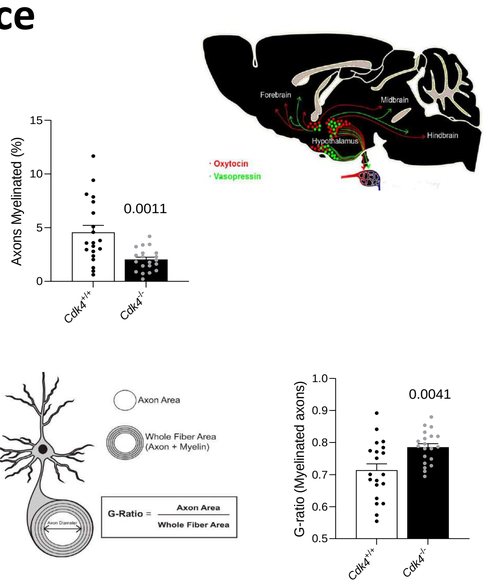
3V: Third Ventricle
ME: Median Eminence



APC (CC-1): oligodendrocyte mature marker

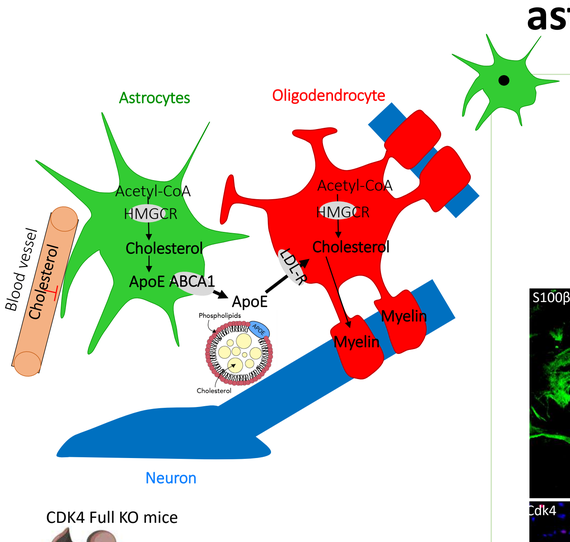
III- Cdk4 deletion impacts the myelin sheath ultrastructure of axons of Oxytocin and AVP magnocellular neurons in the internal median eminence



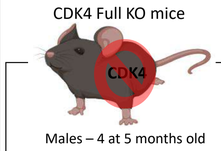


3V: Third Ventricle
ME: Median Eminence

IV- Cdk4 deletion impact cholesterol metabolism in hypothalamic astrocytes

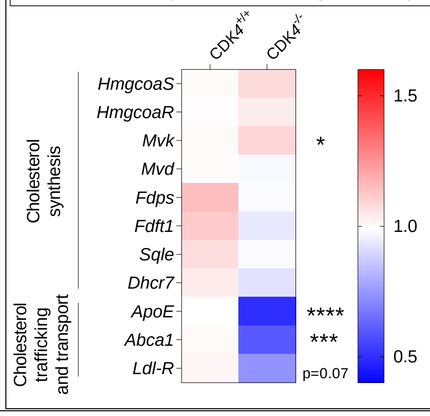


CDK4 Full KO mice

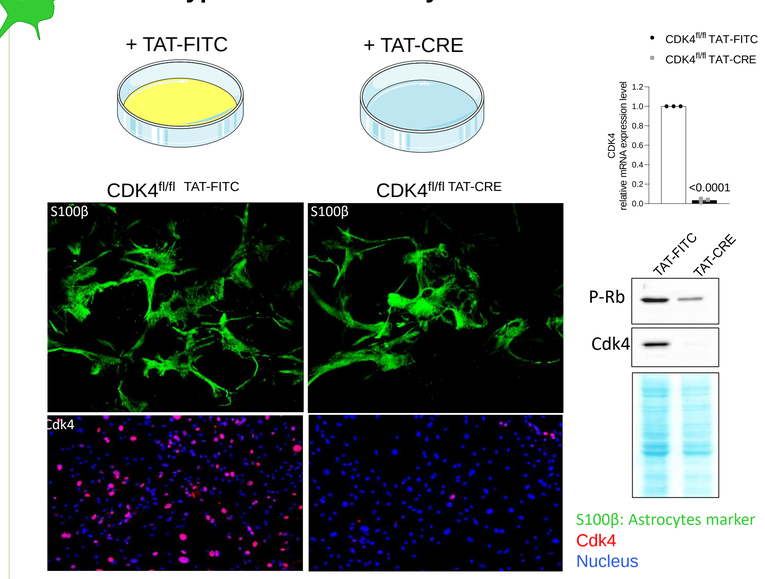


Males – 4 at 5 months old

Relative mRNA expression Cholesterol biosynthesis, trafficking and transport

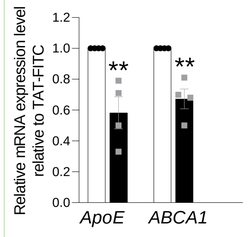


Hypothalamic Astrocytes CDK4$^{fl/fl}$

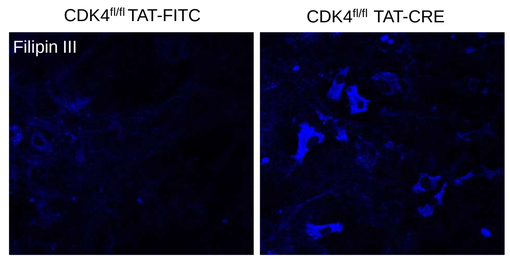


S100β: Astrocytes marker
Cdk4
Nucleus

Relative mRNA expression Cholesterol transport



Cholesterol accumulation in astrocytes cells in cultures



Filipin : Cholesterol probe

N=2 Independent cultures

Conclusion and perspectives

Mouse deleted for CDK4 present default of myelin sheath of hypothalamic neurons such as oxytocin and AVP magnocellular neurons. The alteration of myelin sheath does not seem due to a default of oligodendrocytes ontogenesis but could be due to a disruption of cholesterol transport from astrocytes to oligodendrocytes. Myelin formation and maintenance involve lipid and cholesterol metabolism of glial cells. As CDK4 is described as a cellular metabolism regulator in peripheral tissues, we currently explore the role of CDK4 in the lipid metabolism regulation of hypothalamic glial cells. We also generate mice deleted for CDK4 specifically in glial cells to determine if this phenotype involves a cell-autonomous mechanism or cell-cell interaction.

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